into three groups, control group, H/R group and PD150606 plus H/R group (PD150606 group). To achieve a suitable simulated H/R, cardiomyocytes were incubated with serum-free and glucose-free medium under conditions of hypoxia for 20 min, then they were returned to a normoxic environment with the normal culture medium at 37°C for 2 hours. The viability of cultured cardiomyocytes was examined using trypan blue exclusion test. The cytosolic cytochrome c levels were determined using an ELISA kit. The fluorimetric caspase-3 kit was assayed to measure its activity in the cardiomyocytes. The Suc-LLVY-AMC was used as a fluorescence substrate to measure of calpain activity of cardiomyocytes. Formation of 2-hydroxyethidium (2-EOH) in cardiomyocytes was measured by HPLC to quantify superoxide production.

RESULTS After subjected to I/R, the viable rod-shaped cells were decreased to 37.8% in I/R group when compared with the control group (75.6%), while the calpain inhibitor PD150606 prevented 15% of the rod-shaped cells against H/R-induced cell death. Similarly, compared to I/R group, PD150606 inhibited myocardial apoptosis by decreasing caspase-3 activity 65% and cytochrome c release 47%. Calpain activity was increased 58% in the H/R-cultured cardiomyocytes, yet blunted to 23% by PD150606. Compared to control group, the superoxide (2-EOH) was overproduced 5.43-fold and 4.22-fold, respectively, in I/R and PD150606 group. 2-EOH was partially prevented by PD150606.

CONCLUSIONS Calpain inhibitor PD150606 can effectively reduce H/R injury by reducing oxidative stress mediated myocardial apoptosis. It is plausible that calpain inhibition would be an effective method for protecting the cardiomyocytes from H/R injury.

GW26-e2452

A Study in Construction of Short Hairpin Small Interfering RNA Expression Vector Target Lectin Like Oxidized Low Density Lipoprotein Receptor-1 Gene and Its Effect on foam cells

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OBJECTIVES To construct the short hairpin small interfering RNA(shRNA) eukaryotic expression vector specific to mouse lectin like oxidized low density lipoprotein receptor 1(LOX-1) Gene and to observe its silencing effect on LOX-1 in RAW264.7 cells.

METHODS (1) The pLOX-1-shRNA expression vector was constructed by gene recombination, Then transfected into the cultured RAW264.7 cells. At 48 h after Transfection, the expression of LOX-1 mRNA in RAW264.7 cells were determined by semi-quantitative RT-PCR, the expression of LOX-1 proteins examined by Western blot.

(2) Oil Red O Dyeing experiment was used to show the cellular lipid droplets in lipid-loaded cells. The method of cholesterol oxidase analysis was performed to determine the content of cellular cholesterol. the ability of uptake Dil-ox-LDL in RAW264.7 cells was assayed by fluorescence microscopy.

RESULTS pLOX-1-shRNA expression vector was successfully constructed. Transfection of pLOX-1-shRNA expression vector into RAW264.7 cells down regulated the expression level of LOX-1 gene, as compared with the control Group, transfection of the RAW264.7 cells with LOX 1-shRNA led to a remarkable reduction of the number macrophages transformed into foam cell, and could suppress the uptake of ox-LDL.

CONCLUSIONS The pLOX-1-shRNA expression vector can inhibit The expression of LOX 1 in RAW264.7 cells and the transformation of the macrophages into foam, which may he beneficial in searching new gene therapy of atherosclerosis.

GW26-e2469

Study on Anti-atherosclerosis Mechanism of Yang Xin Shi

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OBJECTIVES To provide experimental basis for revealing the mechanism of yang xin shi in prevention and treatment of coronary heart disease ,we observe its effects on macrophage inflammatory activation and polarity switch by culturing the human monocytic cell line THP-1 with Yang Xin Shi.

METHODS Human THP-1 cells were differentiated into macrophages by the addition of 160 nmol/l phorbol 12-myristate 13-acetate(PMA) for 24 h. We treated macrophages with different concentrations of yang xin shi (10 mg/L, 50 mg/L, 100 mg/L, 200 mg/L) for 12 h, then we stimulated these cells by OX-LDL and detected the secretion of monocyte chemotactic protein 1 (MCP-1) and macrophage migration inhibition factor (MIF) by Enzyme-linked immunosorbent assay (ELISA), and the membrane molecule CD16, CD68 were tested by flow cytometry.

RESULTS 1. Yang xin shi could inhibit the expression of MCP-1 and MIF from the cell culture supernatants as a dose-dependent manner. With the increasing concentrations of Yang xin shi, the secretion of MCP-1 (control: 33.30±2.37 pg/mL; 10 mg/mL: 26.78±1.48 pg/mL; 50 mg/L:25.73±2.25pg/mL;100 mg/L:9.95±2.09pg/mL; 200mg/L: 8.53±1.37pg/mL) and MIF (control: 18.65±0.15 ng/mL; 10 mg/L: 15.50±0.27ng/mL; 50 mg/L: 9.07±0.26ng/mL; 100 mg/L: 4.85±0.12ng/mL; 200mg/L: 4.58±0.36ng/mL) decreased. The difference was statistically significant.

2. Yang xin shi could change the expression of CD16 and CD68 as a dose-dependent manner, the mean fluorescence intensity (MFI) of 100mg/L group could significantly up-regulated the expression of CD16, CD68 (CD16: 96 vs 71.2; CD68: 91.6 vs 54.7). As the concentration increased, the expression of CD16, CD68 were down-regulated in 200mg/L group (CD16:80.6,CD68:72.1).

CONCLUSIONS Yang xin shi may execute its anti-atherosclerotic effect by inhibiting macrophage inflammatory activation and affecting polarity switch.

GW26-e2492

Dynamic research on the heart failure model caused by transverse aortic constriction in Kunming mice

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OBJECTIVES Transverse aortic constriction is widely used to induce heart failure by increasing afterload. It was believed that different pathological and functional changes may appear during a long period after TAC surgery. But comprehensive and dynamic researches about TAC model were still limited. In this study, we try to explore these dynamic pathological and functional changes in TAC mice by multi-measurement during 16 weeks.

METHODS 150 healthy KM mice were divided into two groups randomly: TAC (operated with TAC) group (n=90) and Sham-operation (SH) group (n=60). Pathological, functional detection was performed at 6 different time-points, including 2 weeks, 4 weeks, 6 weeks, 8 weeks, 12 weeks and 16 weeks after surgery. Symptoms and signs of animals were observed and recorded. Echocardiography was used in evaluation of cardiac function and structure by detecting left ventricular mass (LVM) and left ventricular mass index (LVMI), measuring the thickness of anterior or posterior wall, the diameter and volume of left ventricular at both systolic and diastolic stage and analyzing left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS). After gross anatomy and general observation of heart, the heart was weighed and heart/body index was calculated. Haematoxylin-eosin stain and Masson stain were performed to show the histological of heart.

RESULTS (1) Compared with SH, LVM, LVMI and heart/body index of TAC group increased significantly ($P < 0.05$, $P < 0.01$) from 2 to 16 weeks after surgery. LVEF started to reduce after 6 weeks ($P < 0.05$, $P < 0.01$). LVFS started to reduce significantly after 4 weeks ($P < 0.05$, $P < 0.01$). The thickness of left ventricular wall started to thicken significantly after 2 weeks ($P < 0.05$, $P < 0.01$). Left ventricular end-systolic volume and left ventricular internal diameter (systolic) started to increase significantly after 6 weeks ($P < 0.05$, $P < 0.01$). Left ventricular internal diameter (diastolic) and left ventricular end-diastolic volume started to reduce significantly after 12 weeks ($P < 0.05$, $P < 0.01$).

(2) Heart tissue morphology showed vessel wall thickening after 2 weeks. Myocardial hypertrophy appeared after 4 weeks. Lesions aggravated with widened myocardial fiber gap after 12 weeks.

(3) Manifestations of pulmonary congestion were visually observed at each time point. Animals of TAC group were observed with locomotor activity decrease after 4 weeks, swollen soles after 8 weeks, and respiratory distress followed by sudden death after 12 weeks.

CONCLUSIONS Transverse aortic constriction could successfully induce elevated afterload of KM mice and cause Myocardial Hypertrophy/Heart failure in mice. From 2 to 8 weeks after surgery, left ventricular hypertrophy appeared in the TAC mice. According to clinical judgment in the diagnosis of congestive heart failure, this period belonged to pre-clinical stage of heart failure. Similarly,

12 weeks after surgery belonged to clinical stage of heart failure, and 16 weeks after surgery belonged to refractory stage of heart failure.

GW26-e2500

Effect of Salidroside on NRF-1 and NRF-2 of Rats Myocardial after Acute Exhaustive Exercise at Different Time

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OBJECTIVES Nuclear respiratory factors NRF-1 and NRF-2 are the key factors of the regulation of mitochondrial biogenesis. Through the establishment of a single bout of exhausted swimming model, our work is to research the protein and gene expression of mitochondrial biogenesis related factors, NRF-1 and NRF-2, at different time after exhaustive exercise. In order to investigate the effects of exhaustive exercise on NRF-1 and NRF-2, and the effect of Salidroside (SAL) of rats after acute exhaustive.

METHODS A total of 80 health male Sprague Dawley rats (average weight (120 ± 20g))were randomly divided into 10 groups(n=8 in each group), including sedentary control group, exhausted exercise groups (0, 6, 12 and 24 hours after exhausted exercise), SAL and exhausted exercise groups (0, 6, 12 and 24 hours after exhausted exercise). Sedentary control group and exhausted exercise groups were administered with saline (10ml/kg) intragastrically for 14 days. SAL and exhausted exercise groups were administered with SAL (300mg/kg) intragastrically for 14 days. Then exhausted exercise model was established. Exhaustive exercise model is a single bout of exhausted swimming model, according to Thomas standards. Gene expression levels and protein expression of NRF-1, NRF-2 protein were detected.

RESULTS 1. The gene expression of NRF-1 of other groups were significantly higher than that of control group($P < 0.05$), and 6h groups was the highest. With the same time of exhaustive group, the levels of each SAL group NRF-1 mRNA increased significantly ($P < 0.05$).

2. In addition to exhaustive 24h group after exhausted exercise, the gene expression of NRF-2 of other groups were significantly higher than that of control group($P < 0.05$), and 6h groups was the highest. With the same time of exhaustive group, the levels of each SAL group NRF-2 mRNA increased significantly ($P < 0.05$).

3. Compared with control group (0.8574 ± 0.0180), level of the protein expression of NRF-1 in rats myocardial of 0h (0.7313 ± 0.0343), 6h (0.7132 ± 0.0377) and 24h (0.5910 ± 0.0404) after exhausted exercise, SAL and 0h (0.6135 ± 0.0384), 12h (0.5646 ± 0.0217), 24h (0.5617 ± 0.0283) after exhausted exercise were Significantly lower($P < 0.05$).

4. Compared with control group (0.7523 ± 0.0333), level of the protein expression of NRF-2 in rats myocardial of SAL and 6h (1.0295 ± 0.0506), 12h (1.1343 ± 0.0632), 24h (0.8346 ± 0.0371) after exhausted exercise were Significantly higher ($P < 0.05$). 0h (0.3624 ± 0.0532), 6h (0.6601 ± 0.0231), 24h (0.6752 ± 0.0337) after exhausted exercise, SAL and 0h (0.6020 ± 0.0173) after exhausted exercise were Significantly lower($P < 0.05$).

CONCLUSIONS Salidroside can promote the expression of NRF-1, NRF-2 mRNA and the expression of NRF-2 protein of rats myocardial after exhausted exercise.

GW26-e2946

Increased expression of mitochondrial calreticulin in a rat model of dilated cardiomyopathy

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OBJECTIVES Calreticulin (CRT) is involved in the progress of dilated cardiomyopathy (DCM), but the underlying mechanism is still unknown. Since mitochondria have a key role in the progress of DCM, the present study analyzed whether CRT is localized at mitochondria of cardiomyocytes and whether such localization is affected under DCM.

METHODS The DCM model was generated in rats by the daily oral administration of furazolidone for thirty weeks. Echocardiographic and hemodynamic studies were used to assess cardiac function. The location of CRT and its expression were studied by immuno-electron microscopy and western blot. The myocardial apoptosis and mitochondrial enzyme activities were further studied.

RESULTS Echocardiographic and hemodynamic studies demonstrated enlarged left ventricular dimensions and reduced systolic and diastolic function in DCM rats. Immuno-electron microscopy and western blot showed that CRT was present in cardiomyocyte mitochondria and the mitochondrial content of CRT was increased in DCM hearts ($P<0.05$). Morphometric analysis showed notable myocardial apoptosis and mitochondrial swelling with fractured or dissolved cristae in the DCM hearts. Compared with the control group, the mitochondrial membrane potential level of the freshly isolated cardiac mitochondria and the enzyme activities of cytochrome c oxidase and succinate dehydrogenase in the model group were significantly decreased ($P<0.05$), and the myocardial apoptosis index and the caspase activities of caspase-9 and caspase-3 were significantly increased ($P<0.05$). Pearson linear correlation analysis showed that the mitochondrial content of CRT had negative correlations with the mitochondrial function, and a positive correlation with myocardial apoptosis index ($P<0.001$). The protein expression level of cytochrome c and the phosphorylation activitiy of STAT3 in the mitochondrial fraction were significantly decreased in the model group compared with the control group ($P<0.05$).

CONCLUSIONS These data demonstrate that CRT is localized at cardiomyocyte mitochondria and its mitochondrial content is increased in DCM hearts.

GW26-e2962

Continuous Angiotensin-(1-7) infusion improves myocardial calcium transient and calcium transient alternans in ischemia-induced cardiac dysfunction rats

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OBJECTIVES The effects of Ang-(1-7) on calcium homeostasis in dysfunctional cardiomyocytes have not been fully elucidated. The aim of this study was to evaluate the impact of Ang-(1-7) on calcium transient (CaT) in cardiomyocytes during the pathogenesis of heart failure.

METHODS Cardiac dysfunction was induced by ligation of left anterior descending (LAD) coronary artery in adult SD rats. Randomly selected rats were ligated and continuously infused with Ang-(1-7) [HF+Ang-(1-7) group] or saline(HF+saline group) via osmotic minipumps. Sham-operated group without ligation of LAD was also included. After 28 days, hemodynamic parameters were assessed and left ventricular myocytes were isolated. The CaT, the heart rate threshold of CaT alternans (CaT-Alt) and L-type Ca^{2+} channel current (I_{caL})were recorded or measured.

RESULTS The results showed that continuous Ang-(1-7) treatment could attenuate the impairment of cardiac function following LAD ligation. Compared to the Sham-operated group, the HF+saline group showed a decreased CaT amplitude, and a prolonged 50% and 90% CaT recovery time (all $P<0.05$). However, Ang-(1-7) significantly improved these abnormalities(all $P<0.05$ vs HF+saline group). Moreover, Ang-(1-7) significantly improved these abnormalities (All $p<0.05$ vs HF+saline group). Moreover, the heart rate threshold of CaT-Alt was significantly reduced in HF+saline group, and Ang-(1-7) partly restored it ($P<0.05$ vs HF+saline group). Although the ICaL reduced in dysfunctional myocytes, Ang-(1-7) had no effects on it.

CONCLUSIONS Ang-(1-7) attenuates CaT disturbance and increases the heart rate threshold of CaT-Alt during the pathogenesis of HF. These effects contribute to its benefits on improving contractile dysfunction preventing the incidence of arrhythmia in dysfunctional myocytes.

GW26-e3512

Tong Xinluo regulated gene transcription in human umbilical vein endothelial cells injured by homocysteine

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OBJECTIVES To identify key genes differentially expression in the human umbilical vein endothelial cells (HUVECs) injured by homocysteine(HCY) and the protective effect of TXL.

METHODS Human umbilical vein endothelial cells cultivated *in vitro* were divided into three groups: normal group, HCY group (treated with HCY 5mmol/ml 48hours) and TXL group (treated with HCY 5mmol/ml and TXL supermicro powder 5mg/ml 48hours). The total RNA extracted from HUVECs was assessed for differential expression